An introduction to point-of-care testing in extracorporeal circulation and LVADs

Rachel Sara Bercovitz

Division of Hematology/Oncology/Stem Cell Transplant, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL; and Ann & Robert H. Lurie Children's Hospital, Chicago, IL

There is a delicate balance between bleeding and clotting in patients on circuits such as ventricular assist devices or extracorporeal membrane oxygenation. Traditional coagulation tests, prothrombin time, activated partial thromboplastin time, and anti-factor Xa levels, are used to monitor patients on these devices. However, turnaround times and inability to assess global hemostasis, including platelets and fibrinogen have contributed to a recognition that faster, accurate, and more informative coagulation tests are needed. Activated clotting time is used to monitor heparin in patients on circuits and has the advantages of being a near-patient point-of-care test. However, its utility is limited to heparin monitoring. Viscoelastic tests (thromboelastometry and thromboelastography) are global, whole-blood coagulation tests, and whole-blood platelet aggregometry evaluates platelet function. Ideally, these tests can ensure that patients are within the therapeutic range of their antithrombotic medications, identify patients at risk for hemorrhagic or thrombotic complications, and guide management of acute bleeding complications. This ideal is currently hampered by a lack of studies that delineate clear ranges that are clinically relevant. Future research is needed to better understand the optimal use of point-of-care coagulation testing in patients on extracorporeal circuits and ventricular assist devices.

Learning Objectives

- Gain a general understanding of the point-of-care tests that can be used in patients on circuits
- Identify the role these tests can play in guiding anticoagulation and antiplatelet therapies in patients on circuits
- Assess the utility in using these tests to manage hemorrhagic or thrombotic complications in patients on circuits

Introduction

The use of left ventricular assist devices (LVADs) and extracorporeal membrane oxygenation (ECMO) is expanding among both adult and pediatric patients.[1,2] These devices provide critical support to patients with life-threatening cardiac (LVADs and ECMO) and pulmonary (ECMO) failure. In the setting of potentially reversible pulmonary failure, ECMO can be used to support patients for whom conventional ventilatory strategies fail.[2] ECMO can also be used in patients with potentially reversible cardiogenic shock (and can provide short-term support) or as a bridge to a more definitive therapy such as LVAD or heart transplant.[2] In some cardiac failure patients for whom transplant is not an option, LVADs are starting to be used as destination therapy.

Anticoagulation is a necessary adjunct to the use of these circuits. The same primary and secondary hemostatic responses that protect us when blood comes into contact with damaged endothelium or a foreign surface are detrimental to ensuring smooth and interrupted blood flow through the circuit. Heparin is the most commonly used anticoagulant in circuits because of its short half-life and easy reversability.[3] Unfractionated heparin (UFH) binds antithrombin (AT) and subsequently activates 2 mechanisms to downregulate procoagulant factors. The AT-heparin complex binds and inactivates both thrombin and activated factor X (Xa). In patients on ventricular assist devices (VADs), antiplatelet medications including acetylsalicylic acid (ASA; cyclooxygenase inhibition), dipyridamole (a phosphodiesterase inhibitor), and clopidogrel (adenosine 5′-diphosphate [ADP] receptor antagonist) are a part of the anticoagulation management plan. For long-term anticoagulation, patients on VADs are frequently transitioned from UFH to either low-molecular-weight heparin (LMWH) or a vitamin K antagonist (VKA).

The role of coagulation testing in patients on circuits is to ensure that patients are within the targeted range of anticoagulation to minimize the risks of bleeding that can occur when patients are overly anticoagulated as well as avoid the thrombotic complications that occur in patients who are not adequately anticoagulated. Point-of-care (POC) testing can provide clinicians with real-time information on which they can base clinical management. Additionally, some of the POC tests can assess platelet function and degree of inhibition caused by specific antiplatelet medications. This article will compare POC tests with traditional coagulation tests and discuss the role POC testing can play in clinical decision-making and whether POC testing improves patient outcomes.

Standard coagulation tests

The prothrombin time (PT) and activated partial thromboplastin time (aPTT) are plasma-based tests of coagulation.[4,5] Both of these tests are run using platelet-poor plasma made from citrated whole blood

Conflict-of-interest disclosure: The author declares no competing financial interests.

Off-label drug use: None disclosed.

and activating reagents. Although different manufacturers may include different activators, in general, the aPTT reagent contains calcium, phospholipid, and a contact pathway activator (silica, celite, kaolin, ellagic acid, polyphenolic acid)[5]; the PT reagent contains calcium and thromboplastin.[4] Following addition of the reagents, time to clot formation is measured. Clot formation can be detected through optical means with respect to a change in opacity or mechanically with respect to a change in consistency of the reaction mixture.[4,5] Both the PT and aPTT can be prolonged by deficiencies in factors in the common pathway: I, II, V, and X. Prolongation of the aPTT can be due to deficiencies in factors in the contact pathway such as high-molecular-weight kininogen; prekallikrein; and factors VIII, IX, XI, and XII. It can also be prolonged by the presence of UFH or lupus anticoagulants.[5] PT measures activity of the tissue factor pathway and isolated PT prolongation is specific for factor VII deficiency.[5] The international normalized ratio (INR) was developed to account for differences between laboratory PT reagents and standardize VKA therapy monitoring.

The anti-factor Xa (anti-Xa) assay measures the inhibition of activated factor X by the AT-heparin (UFH or LMWH) complex.[6] Like the PT and aPTT, the anti-Xa assay is run using citrated platelet-poor plasma. The anti-Xa assay is specific to the effect of heparinoids and is not affected by deficiencies in other coagulation factors. Some laboratories perform the 2-stage anti-Xa assay in which excess AT is added to the patient sample making heparin the rate-limiting reagent in the inhibition of Xa, which provides a precise quantification of heparin (either UFH or LMWH) in the patient sample. The 1-stage assay does not add excess AT and provides a more accurate assessment of in vivo anticoagulation because the patient's AT and heparin levels are both rate-limiting reagents in this assay. The disadvantage to the 1-stage method is that it cannot distinguish AT deficiency from insufficient heparin.

The aPTT and anti-Xa levels are directly correlated in patients on UFH (r^2 ranges, 0.55-0.61)[7,8]; however, when comparing therapeutic ranges there can be significant discordance.[7] This discordance is likely due to the fact that anti-Xa levels are specific to the heparin plus or minus AT effect, whereas aPTT can be prolonged by numerous factors that are not associated with heparin effect. Adatya and colleagues found that only 32% of patients on a VAD whose aPTT was between 60 and 100 seconds, which was their therapeutic range, had a corresponding anti-Xa level (using the 1-stage assay) between 0.3 and 0.7.[7] The remaining 68% of patients who had an aPTT between 60 and 100 seconds had anti-Xa levels <0.3. No patient was supratherapeutic according to anti-Xa in the case of aPTT being within or below the therapeutic range. Patients whose aPTT was discordantly prolonged compared with anti-Xa level were more likely to have an INR > 1.5, suggesting that decreased levels of other coagulation factors may have prolonged the aPTT rather than heparin effect.[7] The use of anti-Xa level to monitor UFH in patients on ECMO rather than ACT has been shown to reduce number and volume of blood draws for monitoring purposes, extend time to first circuit change, and reduce transfusions and doses of activated factor VII.[9-11]

Although these tests have a role in monitoring anticoagulation medications, they present several limitations in providing real-time information to clinicians who need to provide timely and targeted therapies to patients, particularly those with bleeding or clotting complications.

Point-of-care tests

Activated clotting time

The activated clotting time (ACT) is a common test to monitor anticoagulation in patients on circuits when continuous heparin is being administered.[12] It was first described by Hattersley in 1966; heparin prolongs the ACT in a dose-dependent manner.[13] The ACT is a whole-blood test that uses an activator, usually celite, kaolin, or glass beads, to initiate clotting via the contact pathway, with many advantages over laboratory-based coagulation tests such as the PT and aPTT: as a whole-blood test, the ACT can be performed on a bedside machine, requires a small sample volume, and can be performed by nonlaboratory personnel.[12] The ACT is indicative of inhibition in the contact and or common pathways. Therefore, like the aPTT, although it is sensitive to the effects of heparin, it loses specificity as factors other than heparin can lead to prolongation such as hypothermia, coagulation factor deficiencies, and hemodilution.

Viscoelastic testing

Viscoelastic (VE) testing, first developed in 1948, is a general term for various commercially available tests that use whole blood to derive a number of parameters pertaining to the quality of thrombus formed over time.[14] Thromboelastography (TEG) and rotational thromboelastometry (ROTEM; TEM International GmbH, Munich, Germany) are the 2 most commonly used tests and are both marketed as POC tests.[15] In general, the strength of the clot formed is measured by a torsion wire or pin. In TEG, the wire remains stationary while the cup oscillates through 4°45′ every 5 seconds; in ROTEM, the cup remains stationary while the pin oscillates 4°75′ every 6 seconds.[16] As the clot forms and strengthens, the oscillations are impeded and both the TEG and ROTEM systems detect and translate change in oscillations into the various measurements analyzed. Due to the freestanding wire used in the TEG system, this test is sensitive to vibration, which can produce inaccurate results, making it less suitable for use in the operating suite or at the patient's bedside.[15-17] Both TEG and ROTEM display results both locally and remotely, real time, as the clot is being formed in the test, enabling clinicians to act on these results as they become available. Depending on the system used and the coagulation parameter being measured, results can be available within 5 minutes (for measurements of coagulation factors) whereas others can take longer (usually 15-30 minutes for measurement of platelet function and up to 60 to 90 minutes for completion of fibrinolysis).[15-17] Table 1 shows the different coagulation parameters measured by TEG and ROTEM.

Various reagents in both TEG and ROTEM can evaluate both the contact and tissue factor pathways. Both TEG and ROTEM have heparinase-containing reagents for use in patients on heparin. Obviously, these heparinase-containing tests cannot be used to measure heparinization but can remove the heparin effect to evaluate the other clotting factors. The maximal clot firmness (MCF; ROTEM) and maximum amplitude (MA; TEG) are affected by platelet quality and quantity, fibrinogen, and factor XIII. A study of neonates on cardiopulmonary bypass found that Extem A10 (clot firmness 10 minutes after clotting time [CT], an early and accurate predictor of Extem MCF 0.99) correlated with platelet count ($R = 0.89$) and that A10 of >46.5 had a 88% sensitivity and specificity for identifying patients with platelet count $>100\,000$ platelets per microliter.[18] Discriminating between decreased platelet function and decreased platelet count based on MA or MCF alone is nearly impossible, though it is thought that ROTEM and TEG are more sensitive to platelet count than function. In vitro, platelets inhibited by abciximab (a monoclonal

Table 1. Coagulation parameters measured by viscoelastic testing

Coagulation parameter measured	ROTEM	TEG
Coagulation proteins (contact and/or tissue factor pathways)	Clotting time	R value (reaction time)
Thrombin and fibrin generation; fibrin cross-linking	α angle and clot formation time	K value and α angle
Platelet binding of fibrin and cross-linking by factor XIII	Maximum clot firmness	Maximum amplitude
Fibrinolysis	Clot lysis	LY30 (lysis at 30 min)

antibody that blocks glycoprotein IIbIIIa) showed decreased clot strength compared with noninhibited platelets; however, a sample with a platelet count of ~225 000 platelets per microliter inhibited by abciximab had approximately the same MA as a sample of noninhibited platelets at a concentration of 40 000/μL.[19]

TEG also has platelet mapping (TEG-PM) reagents, which evaluates clot formation using ADP or arachidonic acid (AA) to assess platelet responsiveness in patients on $P2Y_{12}$ inhibitors or cyclooxygenase inhibitors, respectively.[15] ROTEM does not have the equivalent reagents, but does include a nonactivated test that can ostensibly be customized to mimic TEG-PM. Additionally, ROTEM has a reagent (Fibtem; TEM International GmbH) that contains cytochalasin D, a platelet inhibitor, which distinguishes the contribution of platelets from that of fibrinogen to the clot strength. The Fibtem MCF is specific for the contributions of fibrinogen and factor XIII, whereas the Pltem (TEM International GmbH) MCF (Extem MCF − Fibtem MCF; TEM International GmbH) is specific for platelet contributions to the clot strength.[18,20]

Whole-blood platelet aggregometry

Whole-blood aggregometry (WBA), or impedance aggregometry, has 2 electrodes and an electrical current flows between them. Whole-blood samples anticoagulated with either sodium citrate or hirudin are stirred and warmed to 37°C. After a platelet agonist is added, the platelets activate and aggregate to each other and the electrodes, thereby impeding the flow of the electrical current.[21] This impedance is measured by the instrument and translated into "aggregation units," which are measured over time. The area under this curve (AUC) encompasses the lag time until aggregation begins, maximum velocity, and maximum aggregation units and is considered the most clinically relevant predictive value.[22] Agonists such as ADP or AA can detect inhibition by antiplatelet medications.[22,23] Heparinization up to a concentration of 20 U/mL has no effect on WBA measurements; however, protamine appears to reduce aggregation.[24,25] It is uncertain whether protamine reduces aggregation both in vitro and in vivo or whether the highly positively charged peptide interferes with the electrical measurements used by the test.[25,26]

Guiding anticoagulation and antiplatelet therapies

Anticoagulation strategies for patients on ECMO differ from those for patients on VADs. In patients on VADs, the balance between bleeding and clotting is a difficult one to achieve.[27] Most antithrombotic protocols for patients on VADs include an anticoagulant (UFH, LMWH, or warfarin) and antiplatelet therapy (ASA monotherapy in adults or ASA and dipyridamole and/or clopidogrel in pediatric patients). Unfortunately, with many of these medications, a one-size-fits-all dosing regimen does not work, and doses need to be titrated based on response. The majority of trials in adult patients use aPTT to monitor UFH, anti-Xa levels to monitor LMWH, and INR to monitor warfarin.[28] In most adult trials, antiplatelet medications were not adjusted based on platelet function testing.[28,29] In pediatric patients, the most commonly used protocols use standard testing, anti-Xa level, or aPTT to monitor UFH, or alternatively suggest maintaining a TEG kaolin R time between 8 and 15 minutes.[30-32] It also uses the TEG MA as a threshold for starting antiplatelet medications as well as TEG-PM to adjust the dose to ensure at least 70% inhibition with AA and a G (measure of clot strength) <8 in response to ADP.[30,31]

A small, retrospective study of 9 pediatric patients on VAD support compared TEG-PM to WBA to monitor platelet inhibition and found that both testing modalities had high-level variability in AA and ADP inhibition despite steady-state dosing of the medications.[33] Additionally, although this study found a significant correlation between TEG and WBA results in response to AA (correlation coefficient = -0.73; $P = .03$), in patients where the WBA aggregation units (AU) was ~25 (<30 AU is consistent with inhibition),[34] percent inhibition demonstrated by TEG-PM ranged from 0% to 100%.[33]

In 26 adults on long-term, home VAD support, Majeed and colleagues monitored platelet inhibition using both WBA and TEG-PM, defining ASA hyporesponsiveness by a $<50\%$ reduction in maximum impedance or MA, respectively.[35] They found that in 52% of the 656 samples, WBA detected ASA hyporesponsiveness, whereas TEG identified hyporesponsiveness in 10% of these samples.[35] There were 14 thromboembolic events in 8 patients. Six (43%) of these events were in patients who were hyporesponsive to ASA by WBA and 1 (7%) occurred when hyporesponsive as measured by TEG. However, due to the high coefficient of variability in WBA and TEG (57% and 567%, respectively), there was no significant association between thromboembolic event and ASA hyporesponsiveness measured by either instrument.[35]

Karimi et al used TEG to monitor 57 patients on antiplatelet therapy (ASA and dipyridamole in 35 patients, ASA alone in 16 patients) on HeartMate II (Thoratec Corporation, Pleasanton, CA).[36] ASA and dipyridamole were titrated to a TEG-MA of 60 to 70 (normal range, 55-73).[36] Of the 57 subjects, 17 (30%) had bleeding complications and 5 (8.8%) had thromboembolic complications. There was no difference in TEG-MA or INR at times of routine follow-up vs when bleeding or thromboembolic complications developed.[36] However, within their cohort, the late-onset or gastrointestinal bleeding rate was 0.21 events per year compared with an average of 0.49 found in 7 prior published studies, and the authors suggest dose-adjusting antiplatelet medications based on TEG-PM contributed to this lower bleeding rate.[36]

In contrast to the multifaceted antithrombotic approach in patients on VADs, UFH as monotherapy is the most commonly used anticoagulation strategy in patients on ECMO; antiplatelet medications are not used.[3,37] The majority of institutions surveyed use either aPTT (41.7%) or ACT (41.7%) as the primary test to monitor UFH. An additional 10.4% use anti-Xa levels and 8.3% (n = 4) use VE testing.[3] Fifteen of the 48 respondents use VE as their secondary anticoagulation test, and of those 19 institutions, 15 (79%) use TEG instead of ROTEM.[3]

There have been some small studies looking at heparin monitoring using ROTEM or TEG compared with aPTT or anti-Xa, and the

results are mixed. Ranucci and colleagues found that in general, TEG R time did not consistently correlate with aPTT and had poor predictive value with respect to determining whether the aPTT was out of range (54.8% for shortened aPTT and 38.5% for prolonged aPTT).[38] However, in combination, ACT and TEG R time were able to predict whether the patient's aPTT was within therapeutic range (71% positive predictive value for supratherapeutic aPTT and 83% for subtherapeutic aPTT).[38] Panigada and colleagues compared adjusting heparin dosing based on TEG R time vs aPTT.[39] They found that patients monitored by TEG had more frequent heparin infusion changes and spent less time within the therapeutic range.[39] There was no difference in the incidence of thrombotic complications (19% in each cohort). There was a nonstatistically significant trend toward increased bleeding incidence in patients monitored using aPTT (71.4% vs 47.6%; $P = .21$).[39] A retrospective study of using ROTEM (specifically, the Intem [TEM International GmbH] CT and clot formation time [CFT]) and aPTT to monitor heparinization in patients on ECMO found that the Intem CT was frequently within the normal range despite escalating doses of heparin and increasing aPTT and ACT.[40] The authors expressed their concern that the use of Intem CT alone could lead to excessive UFH exposure.[40]

Based on these studies, there appears to be significant intrasubject variability with respect to the TEG and ROTEM measurements despite patients being on a consistent anticoagulation or antiplatelet dose, which may limit its clinical applicability. In individual patients, there was not a correlation between TEG, INR, or aPTT and bleeding or thromboembolic complications, in part due to this variability.[35,36] To date, there has not been a study published that demonstrates ROTEM or TEG to be superior to aPTT or ACT, though advantages of VE testing, including the ability to monitor hemostatic potential of platelets and fibrinogen, may make it a favorable option if noninferiority with respect to bleeding and thrombotic complications can be demonstrated.

Predicting and responding to hemorrhagic or thrombotic complications

The rapid turnaround time of POC testing is appealing because it provides clinicians with prompt information so as to provide timely and targeted therapy to treat or prevent hemorrhagic and thrombotic complications while on VADs or ECMO. The goal of POC-guided algorithms is to reduce blood product use, by both minimizing unnecessary platelet and plasma transfusions as well as resolving hemorrhage more quickly to reduce red blood cell (RBC) requirements.[41,42]

Unlike surgical POC-guided algorithms, those used in patients on VADs or ECMO would be ideally able to identify patients who are at risk of thrombosis as well as hemorrhage.[43] One major problem with using POC testing, including TEG, ROTEM, and WBA, is that there are no unequivocal ranges that predict who are the patients at risk of bleeding, who are at risk of thrombosis, and who are within the desired therapeutic range of their antithrombotic medications.[43]

The flow dynamics of circuits can lead to loss of high-molecular-weight multimers (HMWMs) of von Willebrand factor (VWF), leading to an acquired von Willebrand disease (VWD) that mimics 2A and can increase bleeding risk.[44] Both TEG (clotting index [CI]) and WBA (ristocetin-induced platelet aggregation) may be sensitive, and specific tests that can distinguish patients with congenital type 2 VWD from healthy controls[45,46]; however, these tests have not been used to identify patients with circuit-related acquired VWD. Evaluation of VWF antigen, activity, and multimers remains the gold standard for identifying acquired VWD is patients on VADs or ECMO.

There is a fine balance between bleeding and clotting in patients on VADs and ECMO and a goal of using POC testing is to predict patients who are at risk of bleeding and clotting. Thus far, the studies are mixed with some showing that decreased function as measured by VE testing is associated with bleeding in patients on VADs and ECMO,[47,48] whereas others found no predictive value in VE testing parameters.[49,50] In a study of 382 adults who underwent implantation of a VAD, a subtherapeutic INR (<2) was associated with increased risk of pump thrombus[51]; however, similar studies using VE testing or WBA parameters have not been performed.

Summary

Both bleeding and thrombotic complications are common in patients on VADs and ECMO. Laboratory monitoring of anticoagulation and antiplatelet medications is used to ensure patients are in the therapeutic range, which will ostensibly reduce risk of bleeding due to over anticoagulation and clotting due to under anticoagulation. With the goal of identifying patients at risk of either bleeding or thrombotic complications, near-patient testing with a rapid turnaround time is an optimal solution. There are some institutions that believe in the value of ROTEM, TEG, or WBA and use these tests in a variety of clinical scenarios; however, to date, there have been no randomized, multi-institutional trials comparing TEG, ROTEM, or WBA with aPTT, PT/INR, or anti-Xa levels for monitoring and dose-adjusting anticoagulant and antiplatelet medications in patients on LVADs or ECMO. The use of VE testing, including TEG-PM, is more established and integrated into the anticoagulation protocols in pediatric patients on LVADs.[30-32]

With a lack of superiority of TEG and ROTEM over standard coagulation tests, POC versions of INR are being developed and studied in other patient populations, such as home warfarin monitoring in the case of POC-INR.[52] Prior studies in patients on cardiopulmonary bypass have shown concordance between near-patient PT/INR testing and central laboratory results, whereas near-patient aPTT testing was discordant.[40,53] Further research is needed to both validate predictive ranges in current POC tests such as TEG, ROTEM, and WBA as well as to develop reliable and accurate POC versions of the standard coagulation tests.

Correspondence

Rachel Sara Bercovitz, Ann & Robert H. Lurie Children's Hospital, 225 E Chicago Ave, Box 30, Chicago, IL 60611; e-mail: rbercovitz@luriechildrens.org.

References

1. Briasoulis A, Inampudi C, Akintoye E, Adegbala O, Alvarez P, Bhama J. Trends in utilization, mortality, major complications, and cost after left ventricular assist device implantation in the United States (2009 to 2014). *Am J Cardiol*. 2018;121(10):1214-1218.
2. Burki S, Adachi I. Pediatric ventricular assist devices: current challenges and future prospects. *Vasc Health Risk Manag*. 2017;13:177-185.
3. Esper SA, Welsby IJ, Subramaniam K, et al. Adult extracorporeal membrane oxygenation: an international survey of transfusion and anticoagulation techniques. *Vox Sang*. 2017;112(5):443-452.
4. Ignjatovic V. Prothrombin time/international normalized ratio. *Methods Mol Biol*. 2013;992:121-129.
5. Ignjatovic V. Activated partial thromboplastin time. *Methods Mol Biol*. 2013;992:111-120.

6. Newall F. Anti-factor Xa (anti-Xa) assay. *Methods Mol Biol*. 2013;992: 265-272.
7. Adatya S, Uriel N, Yarmohammadi H, et al. Anti-factor Xa and activated partial thromboplastin time measurements for heparin monitoring in mechanical circulatory support. *JACC Heart Fail*. 2015;3(4): 314-322.
8. Bhatia AK, Yabrodi M, Carroll M, et al. Utility and correlation of known anticoagulation parameters in the management of pediatric ventricular assist devices. *World J Cardiol*. 2017;9(9):749-756.
9. Niebler RA, Parker H, Hoffman GM. Impact of anticoagulation and circuit technology on complications during extracorporeal membrane oxygenation [published online ahead of print 8 May 2018]. *ASAIO J*. doi: 10.1097/MAT.0000000000000811.
10. Delmas C, Jacquemin A, Vardon-Bounes F, et al. Anticoagulation monitoring under ECMO support: a comparative study between the activated coagulation time and the anti-Xa activity assay [published online ahead of print 1 January 2018]. *J Intensive Care Med*. doi: 10.1177/0885066618776937.
11. O'Meara LC, Alten JA, Goldberg KG, et al. Anti-Xa directed protocol for anticoagulation management in children supported with extracorporeal membrane oxygenation. *ASAIO J*. 2015;61(3):339-344.
12. Horton S, Augustin S. Activated clotting time (ACT). *Methods Mol Biol*. 2013;992:155-167.
13. Hattersley PG. Activated coagulation time of whole blood. *JAMA*. 1966; 196(5):436-440.
14. Shen L, Tabaie S, Ivascu N. Viscoelastic testing inside and beyond the operating room. *J Thorac Dis*. 2017;9(suppl 4):S299-S308.
15. Whiting P, Al M, Westwood M, et al. Viscoelastic point-of-care testing to assist with the diagnosis, management and monitoring of haemostasis: a systematic review and cost-effectiveness analysis. *Health Technol Assess*. 2015;19(58):1-228, v-vi.
16. Zaky A. Thromboelastometry versus rotational thromboelastography in cardiac surgery. *Semin Cardiothorac Vasc Anesth*. 2017;21(3):206-211.
17. Jackson GN, Ashpole KJ, Yentis SM. The TEG vs the ROTEM thromboelastography/thromboelastometry systems. *Anaesthesia*. 2009; 64(2):212-215.
18. Scott JP, Niebler RA, Stuth EAE, et al. Rotational thromboelastometry rapidly predicts thrombocytopenia and hypofibrinogenemia during neonatal cardiopulmonary bypass. *World J Pediatr Congenit Heart Surg*. 2018;9(4):424-433.
19. Noorman F, Hess JR. The contribution of the individual blood elements to the variability of thromboelastographic measures [published online ahead of print 20 September 2018]. *Transfusion*. doi:10.1111/trf.14884.
20. Ji SM, Kim SH, Nam JS, et al. Predictive value of rotational thromboelastometry during cardiopulmonary bypass for thrombocytopenia and hypofibrinogenemia after weaning of cardiopulmonary bypass. *Korean J Anesthesiol*. 2015;68(3):241-248.
21. Multiplate Analyzer. Franklin, OH: Diapharma. https://diapharma.com/product/platelet-function-testing/multiplate-analyzer/. Accessed 29 September 2018.
22. Tóth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. *Thromb Haemost*. 2006;96(6):781-788.
23. Paniccia R, Priora R, Liotta AA, Abbate R. Platelet function tests: a comparative review. *Vasc Health Risk Manag*. 2015;11:133-148.
24. Gertler R, Wiesner G, Tassani-Prell P, Braun SL, Martin K. Are the point-of-care diagnostics MULTIPLATE and ROTEM valid in the setting of high concentrations of heparin and its reversal with protamine? *J Cardiothorac Vasc Anesth*. 2011;25(6):981-986.
25. Olsson A, Alfredsson J, Håkansson E, Svedjeholm R, Berglund J, Berg S. Protamine reduces whole blood platelet aggregation after cardiopulmonary bypass. *Scand Cardiovasc J*. 2016;50(1):58-63.
26. Griffin MJ, Rinder HM, Smith BR, et al. The effects of heparin, protamine, and heparin/protamine reversal on platelet function under conditions of arterial shear stress. *Anesth Analg*. 2001;93(1):20-27.
27. Raffini L. Anticoagulation with VADs and ECMO: walking the tightrope. *Hematology Am Soc Hematol Educ Program*. 2017;2017:674-680.
28. Rossi M, Serraino GF, Jiritano F, Renzulli A. What is the optimal anticoagulation in patients with a left ventricular assist device? *Interact Cardiovasc Thorac Surg*. 2012;15(4):733-740.
29. Maltais S, Kilic A, Nathan S, et al; PREVENT Study Investigators. PREVENtion of HeartMate II pump thrombosis through clinical management: the PREVENT multi-center study. *J Heart Lung Transplant*. 2017;36(1):1-12.
30. Steiner ME, Bomgaars LR, Massicotte MP; Berlin Heart EXCOR Pediatric VAD IDE Study Investigators. Antithrombotic therapy in a prospective trial of a pediatric ventricular assist device. *ASAIO J*. 2016; 62(6):719-727.
31. Almond CS, Buchholz H, Massicotte P, et al. Berlin Heart EXCOR Pediatric ventricular assist device investigational device exemption study: study design and rationale. *Am Heart J*. 2011;162(3):425-435.
32. Rosenthal DN, Lancaster CA, McElhinney DB, et al. Impact of a modified anti-thrombotic guideline on stroke in children supported with a pediatric ventricular assist device. *J Heart Lung Transplant*. 2017; 36(11):1250-1257.
33. Ferguson LP, Duong P, Pearce KF, Murphy P, Biss TT. Monitoring of antiplatelet therapy in children on ventricular assist device support: comparison of multiplate and thromboelastography platelet mapping [published online ahead of print 27 February 2018]. *ASAIO J*. doi: 10.1097/MAT.0000000000000768.
34. Zeymer U, Hohlfeld T, Vom Dahl J, et al. Prospective, randomised trial of the time dependent antiplatelet effects of 500 mg and 250 mg acetylsalicylic acid i. v. and 300 mg p. o. in ACS (ACUTE). *Thromb Haemost*. 2017;117(3):625-635.
35. Majeed F, Kop WJ, Poston RS, Kallam S, Mehra MR. Prospective, observational study of antiplatelet and coagulation biomarkers as predictors of thromboembolic events after implantation of ventricular assist devices. *Nat Clin Pract Cardiovasc Med*. 2009;6(2):147-157.
36. Karimi A, Beaver TM, Hess PJ, et al. Close antiplatelet therapy monitoring and adjustment based upon thrombelastography may reduce late-onset bleeding in HeartMate II recipients. *Interact Cardiovasc Thorac Surg*. 2014;18(4):457-465.
37. Bembea MM, Annich G, Rycus P, Oldenburg G, Berkowitz I, Pronovost P. Variability in anticoagulation management of patients on extracorporeal membrane oxygenation: an international survey. *Pediatr Crit Care Med*. 2013;14(2):e77-e84.
38. Ranucci M, Baryshnikova E, Cotza M, et al; Group for the Surgical and Clinical Outcome Research (SCORE). Coagulation monitoring in postcardiotomy ECMO: conventional tests, point-of-care, or both? *Minerva Anestesiol*. 2016;82(8):858-866.
39. Panigada M, E Iapichino G, Brioni M, et al. Thromboelastography-based anticoagulation management during extracorporeal membrane oxygenation: a safety and feasibility pilot study. *Ann Intensive Care*. 2018;8(1):7.
40. Prakash S, Wiersema UF, Bihari S, Roxby D. Discordance between ROTEM® clotting time and conventional tests during unfractionated heparin-based anticoagulation in intensive care patients on extracorporeal membrane oxygenation. *Anaesth Intensive Care*. 2016;44(1):85-92.
41. Görlinger K, Dirkmann D, Hanke AA. Potential value of transfusion protocols in cardiac surgery. *Curr Opin Anaesthesiol*. 2013;26(2): 230-243.
42. Lier H, Vorweg M, Hanke A, Görlinger K. Thromboelastometry guided therapy of severe bleeding. Essener Runde algorithm. *Hamostaseologie*. 2013;33(1):51-61.
43. Petricevic M, Milicic D, Boban M, et al. Bleeding and thrombotic events in patients undergoing mechanical circulatory support: a review of literature. *Thorac Cardiovasc Surg*. 2015;63(8):636-646.
44. Adatya S, Bennett MK. Anticoagulation management in mechanical circulatory support. *J Thorac Dis*. 2015;7(12):2129-2138.
45. Schmidt DE, Bruzelius M, Majeed A, Odeberg J, Holmström M, Ågren A. Whole blood ristocetin-activated platelet impedance aggregometry (Multiplate) for the rapid detection of von Willebrand disease. *Thromb Haemost*. 2017;117(8):1528-1533.
46. Schmidt DE, Majeed A, Bruzelius M, Odeberg J, Holmström M, Ågren A. A prospective diagnostic accuracy study evaluating rotational

thromboelastometry and thromboelastography in 100 patients with von Willebrand disease. *Haemophilia*. 2017;23(2):309-318.
47. Laine A, Niemi T, Suojaranta-Ylinen R, et al. Decreased maximum clot firmness in rotational thromboelastometry (ROTEM®) is associated with bleeding during extracorporeal mechanical circulatory support [published online ahead of print 28 April 2016]. *Perfusion*. doi:10.1177/0267659116647473.
48. Nair P, Hoechter DJ, Buscher H, et al. Prospective observational study of hemostatic alterations during adult extracorporeal membrane oxygenation (ECMO) using point-of-care thromboelastometry and platelet aggregometry. *J Cardiothorac Vasc Anesth*. 2015;29(2):288-296.
49. Panigada M, Iapichino G, L'Acqua C, et al. Prevalence of "flat-line" thromboelastography during extracorporeal membrane oxygenation for respiratory failure in adults. *ASAIO J*. 2016;62(3):302-309.
50. Hellmann C, Schmutz A, Kalbhenn J. Bleeding during veno-venous ECMO cannot reliably be predicted by rotational thrombelastometry (ROTEM). *Perfusion*. 2017;8;33(4):289-296.
51. Najjar SS, Slaughter MS, Pagani FD, et al; HVAD Bridge to Transplant ADVANCE Trial Investigators. An analysis of pump thrombus events in patients in the HeartWare ADVANCE bridge to transplant and continued access protocol trial. *J Heart Lung Transplant*. 2014;33(1):23-34.
52. Mbokota N, Schapkaitz E, Louw S. Verification of the qLabs international normalized ratio point-of-care device for monitoring of patients attending an anticoagulation clinic. *Int J Lab Hematol*. 2018;40(5):508-514.
53. Despotis GJ, Santoro SA, Spitznagel E, et al. On-site prothrombin time, activated partial thromboplastin time, and platelet count. A comparison between whole blood and laboratory assays with coagulation factor analysis in patients presenting for cardiac surgery. *Anesthesiology*. 1994;80(2):338-351.